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I. Ishiyama,[1] *M.D.; M. Orui,*[1] *Ph.D.; K. Ogawa,*[1] *Ph.D.; and T. Kimura,*[1] *Ph.D.*

The Determination of Isoantigenic Activity from Latent Fingerprints: Mixed Cell Agglutination Reaction in Forensic Serology

In forensic serology, A-B-H blood group substances have been successfully detected by mixed agglutination and absorption-elution techniques. By modifying the methods of Kouvarik et al [*1*] and Davidsohn [*2*], Ishiyama et al [*3*] recently developed a new technique for mixed cell agglutination reaction (MCAR) as follows:

1. Specimens were affixed onto the adhesive side of cellophane tape and then placed on a microscope slide.
2. Anti-A and anti-B agglutinins and *Ulex* anti-H lectin were used for sensitization.
3. The specimens were thoroughly washed to remove nonreactive agglutinins.
4. A corresponding indicator cell aliquot was added (sandwich method).
5. The slide was reversed and immersed in saline so that the nonreactive cells would dissociate spontaneously.
6. The results were read microscopically.

Results were excellent. Samples were not destroyed during collection and processing; morphological and serological examination could be performed simultaneously; and the results were not difficult for an experienced person to read.

Another advantage is that our method can be applied not only to fixed, nonsoluble specimens but also to water-soluble blood group substances without any special treatment. Biological fluids (saliva, semen, and so forth) were placed directly onto the adhesive side of cellophane tape and dried completely with a hair drier. By this simple procedure enough soluble blood group substance can be fixed on the cellophane tape for analysis. Sensitivity of the reaction is several thousand times greater than other routine serological tests, including the absorption-elution test. For example, isoantigenic activity can be determined in saliva diluted $1:10^8$ to $1:10^{10}$ in the secretor and $1:10^4$ to $1:10^5$ in the nonsecretor [*4*]. At the same time, our method gives exact information concerning the topographical distribution of isoantigens. With our technique it is possible to serologically demonstrate a fingerprint pattern impressed on the adhesive side of cellophane tape.

Research was also done on the serological examination of fingerprints on paper [*5–7*]. The latent fingerprints were developed with ninhydrin and affixed to the cellophane tape before the MCAR was performed. The results were excellent in many cases, but occasionally some difficulties were encountered, especially in the process of the dissociation of nonreactive cells. We resolved these difficulties and developed a standard method for

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[1]Department of Legal Medicine, Teikyo University School of Medicine, Tokyo, Japan.

blood group determination from latent fingerprints. These results are described in this paper.

Materials and Methods

Fingerprints were obtained on microscope slides from medical students whose blood types and secretor status were then determined. No strict conditions were followed in sampling. The fingertips were pressed directly on the microscope slide and and the fingerprints were preserved in the slide box until use.

Fingerprints on paper were obtained from several persons with A, B, O, and AB blood types. The fingerprints were placed by touch on papers of various quality (letter paper, typewriter paper, newspaper, toilet paper, and so forth).

Routine methods were used to demonstrate the fingerprints. A latent fingerprint on a microscope slide was detected by embossment with aluminum powder. To detect a fingerprint on paper, the paper was sprayed with an acetone solution containing 3% ninhydrin and kept at 105°C for three min. The ninhydrin-positive part was cut off, photographed, and later examined by MCAR. In some cases the prints, after being stained with ninhydrin and before being examined by MCAR, were divided in half and each part was examined with either anti-A or anti-B agglutinins.

Modified MCAR on adhesive cellophane tape was performed according to the Ishiyama technique [*8*]. Some improvements to the technique were made and are reported in the next section. Results were excellent.

Experimental Results

Latent Fingerprints on Microscope Slides

Swabbing Method—A small piece of absorbent cotton (1 to 2 mg) was taken with a pincette and dipped into a small quantity of 50% ethanol solution (ethanol/water ratio, 1:1). Latent fingerprints were swabbed thoroughly with this cotton. The cotton was then dried completely at room temperature. In some cases, the cotton was dried rapidly by warming it with a hair drier. After drying, the cotton was attached lightly to the adhesive surface and tested by MCAR. The results are shown in Fig. 1. The tip of the pincette was heated to prevent contamination by blood group substances during sampling.

Scraping Method—A fresh commercial safety razor blade was used to collect specimens from the latent fingerprints on the microscope slide. The prints on the surface were scraped tangentially with this blade so that dirt from the prints was transferred to one side of the edge of the blade. The specimen on the blade was affixed directly onto the adhesive surface and then examined by MCAR. The results are shown in Fig. 2.

Minimal Amount of Latent Fingerprints for Serological Investigation—As we found that the latent fingerprints could be analyzed serologically, further experiments were performed to estimate the minimal amount of specimen required for accurate determination of blood group specificity. Using the scraping and swabbing methods, we succeeded in determining the blood type from one epidermal ridge lining of 8 to 15 mm in length. Hence, if a complete pattern of the latent fingerprint is to be examined, serological tests can be performed repeatedly with the same specimens and therefore the determination of the serological properties can be assured.

Serological Investigation of Latent Fingerprints After Replication with Aluminum Powder—In criminal investigations, aluminum powder is often used to detect latent fingerprints. We examined the effect of this procedure on the serological examination.

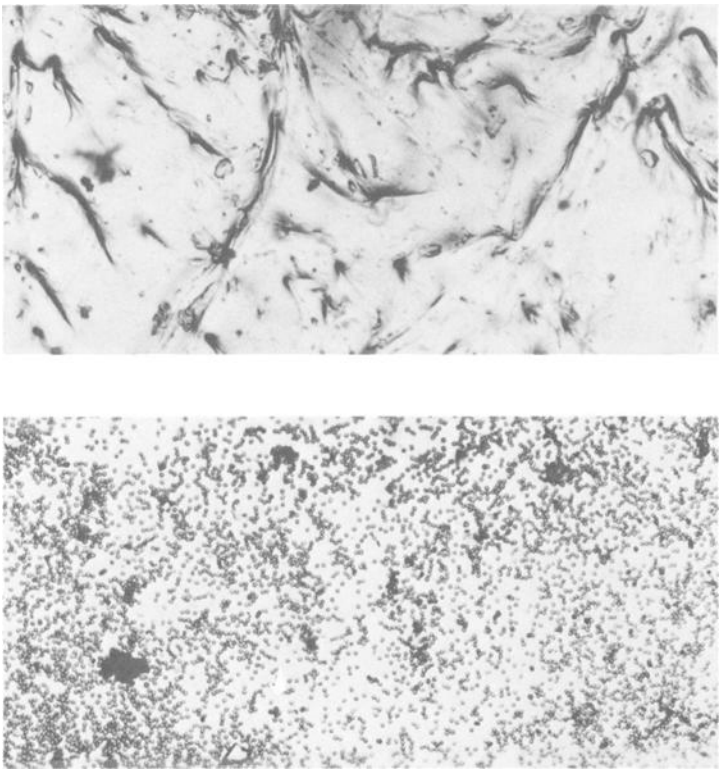

FIG. 1—*Blood group A-B-H determination of latent fingerprints (blood group B, secretor) on a glass wall by modified MCAR (swabbing method);* (top) *specimens on cellophane tape were sensitized with anti-A followed by MCAR with type A cells with negative results;* (bottom) *the same specimens were sensitized with anti-B, followed by MCAR with type B cells with positive results evidenced by the marked segregation of the cells.*

Fingerprints on the microscope slide were first demonstrated by the routine aluminum powder procedure and then replicated on a gelatin plate. Serological examinations were then conducted on the material removed by swabbing and scraping. As shown in Fig. 3, a distinct determination of blood type could be achieved.

H-Activity Problem—One of the most difficult problems in the determination of blood type is the definitive demonstration of H-activity. It is very important to obtain a good quality anti-H lectin, which must be specific and of high titer. In this experiment, we used *Ulex* as the source of anti-H agglutinin. The titer was 1:16 against the intact O-cells. The enzyme treatment inevitably caused marked, nonspecific adhesion. We examined various means to eliminate this difficulty and found that the most effective method was to treat the adhesive surface with saline containing 0.01% Tween 80. After fixation of the specimens, the adhesive surface was washed first with this solution for a short period (30 s) and then with normal saline. This procedure eliminated the non-specific adhesion of enzyme-treated O-cells. After the MCAR was modified in this way, we found no difficulties in determining blood type.

Latent Fingerprints on Paper

Demonstration with Aid of Modified MCAR—Before the serological investigation, the

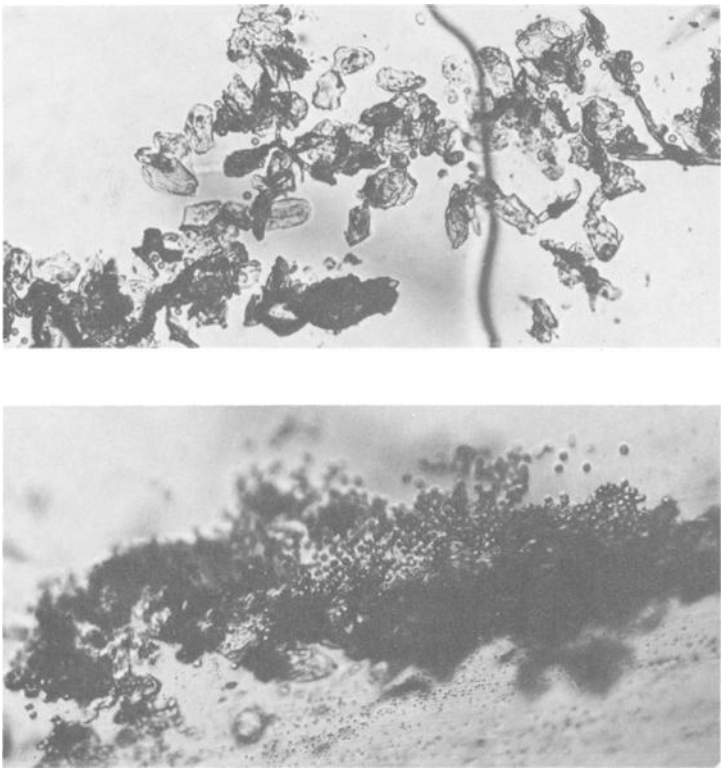

FIG. 2—*Blood group A-B-H determination of latent fingerprints (blood group B, secretor, same sample as in Fig. 1) on glass wall by MCAR (scraping method);* (top) *MCAR is the same as in Fig. 1* (top) *with negative results;* (bottom) *MCAR is the same as in Fig. 1* (bottom) *with positive results evidenced by rosette-like agglutination.*

fingerprints were developed with ninhydrin. Thereafter, the stained part was cut from the paper, affixed to the adhesive tape, and examined serologically by the modified MCAR. As shown in Fig. 4, it was possible in some cases to demonstrate the whole print pattern distinctly. Some difficulties were encountered, especially during the dissociation procedure of the nonreactive cells.

Improvement of Modified MCAR—Inasmuch as we experienced some difficulty in determining the blood type from fingerprints on paper, we improved the method by the following procedures.

The stained part of the paper was cut off, divided in half, and sensitized with anti-A and anti-B reagents, respectively, by adding agglutinins directly to the specimens. After sensitization, the samples were washed thoroughly in a petri dish. (The paper, after sensitization, was immersed in saline and shaken by hand; this washing was continued for 3 min and repeated five times, the saline solution being changed each time.) Even after thorough washing, the paper was stained with acriflavine or trypan blue (which are contained in the standard sera of anti-A and anti-B); this suggests that some nonspecific absorption of agglutinins may occur. A portion (0.1%) of the red cell aliquot was poured into a petri dish to a depth of 2 to 3 mm. The washed sample was immersed in this red cell aliquot at room temperature for 15 min without shaking, during which time the cells settled evenly on the surface of the sample. After this, the petri dish was slightly

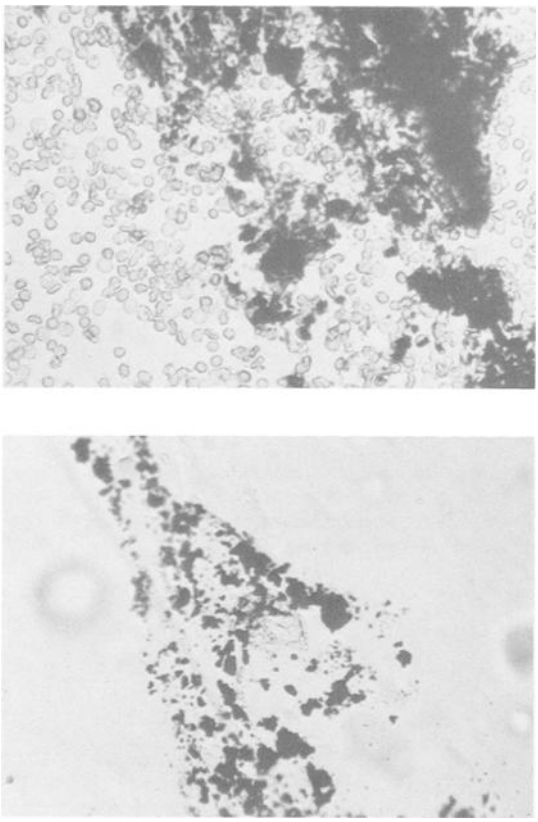

FIG. 3—*Blood group determination of latent fingerprints after the treatment with aluminum-gelatin plate for replication (swabbing). Fingerprints were obtained from a blood group A, secretor-type individual.* (Top) *Sensitization with anti-A, followed by indicator type A cells, gave positive results; black figures and spots show aluminum powder.* (Bottom) *Sensitization with anti-B, followed by indicator B cells, gave negative results.*

shaken by hand, and the cell layer at the bottom was faintly shaken during this procedure. The cells, which were nonreactive, were isolated from the surface and some of them were agglutinated once more with the reacted cells that were arranged along the epidermal ridge lining. The fingerprint pattern was then distinct (Fig. 5).

Thereafter, the solution in the petri dish was discarded by slowly sucking it out with a Pasteur pipette so that no disturbance of serological reaction occurred. After this, absolute ethanol was poured slowly to fix the pattern, and the petri dish was left standing in this state for 5 min. Then the alcohol was discarded and the dish was dried completely at room temperature. In this way a distinct pattern could be prepared, and the fingerprints could be studied for secretor status. In the case of a nonsecretor the reaction was markedly weak and the serological pattern was disturbed during the embossing procedure, but, as shown in Fig. 6, a distinct determination of serological reaction could be made.

We were able to determine serologically latent fingerprints on paper that was fairly impervious to water. Letter paper, typewriter paper, newspaper, and, in some instances, filter paper can be examined by this method. When papers of bad quality are examined, thorough washing is an inevitable requirement.

Papers not impervious to water cannot be examined by this method. We offer the fol-

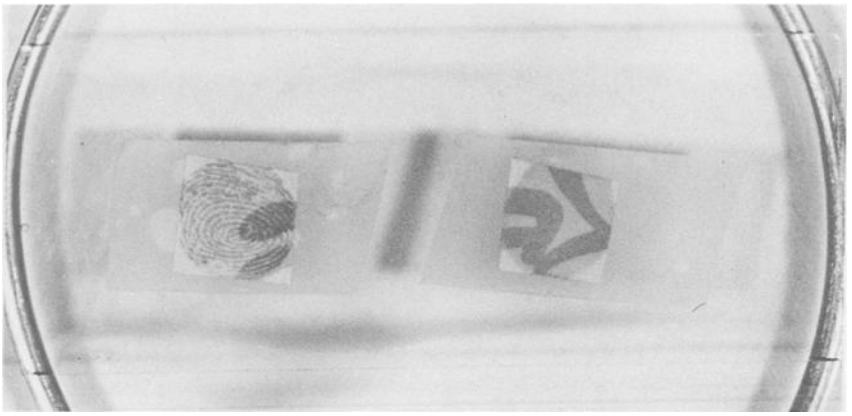

FIG. 4—*Demonstration of fingerprint pattern by modified MCAR:* (left) *sensitized with anti-A, followed by indicator type A cells, with positive results (the whole pattern of fingerprint was demonstrated);* and (right) *sensitized with anti-B, followed by indicator type B cells, with negative results (the spot shows staining with acriflavine, contained in standard anti-B serum).*

FIG. 5—*Improved MCAR for the determination of latent fingerprints on paper (blood type B, secretor);* (left) *ninhydrin staining and* (right) *the MCAR pattern, obtained by improved method. Sensitization was done with anti-B, followed by MCAR with type B cells. The fingerprint pattern was definitely demonstrated by serological technique in comparison with ninhydrin method. No positive reaction (nonspecific adhesion of cells) was observed in cases in which the antigens and sensitizing agglutinins were incompatible.*

lowing procedure for examining fingerprints on such paper: cut the ninhydrin-positive part of the paper into small pieces, affix the pieces to the cellophane tape, and perform MCAR using this specimen. With this procedure we could determine the blood type, irrespective of secretor types, in an examination of the filter part of a cigarette [*1*]. The results are shown in Fig. 7.

Some difficulty is encountered in determining H activity. *Ulex* anti-H lectin is usually so weak that embossing is not possible by using the intact O-cells. Enzyme treatment of the cells increases nonspecific adhesion so that no distinct demonstration of the finger-

FIG. 6—*The MCAR pattern obtained by improved method (blood type A, nonsecretor).* (Left) *The serologically positive pattern, which corresponds to epidermal ridge lining, was demonstrated, but this pattern was disturbed during sucking of aliquot.* (Middle) *No positive pattern was obtained when the antigen and agglutinins were incompatible. Faint epidermal linings were due to ninhydrin staining.* (Right) *Ninhydrin staining.*

prints by serological technique is possible in such cases. Determination of blood type O depends, however, not only on diagnosis by exclusion but also on on applying the modified MCAR on the cellophane tape using a small piece (or several paper fibers) of ninhydrin-positive paper.

Discussion

The modified procedure using MCAR on cellophane tape makes it possible to determine the A-B-H blood types of various specimens which could not be examined before because of technical difficulties. In this sense, serological investigations in forensic science are expanded enormously by this method.

There are several reasons why the determination of blood type from fingerprints is most reliable when the fingerprints remain on paper.

First, blood group active glycoprotein can be made insoluble and fixed during ninhydrin staining (heat and acetone treatments) so that the localization of the replicated epidermal ridge lining is easily demonstrated by the serological test.

Second, the content or concentration of blood type active substance in sweat is assumed to be relatively high (the concentration may be comparable with that of saliva); hence, the demonstration of fingerprint pattern can be done much more easily when traces of sweat that remain on the paper are available, as the topological distribution of blood type active substance along the epidermal ridge lining is possibly the same one, when the undiluted saliva is distributed in a similar pattern.

Third, the affinity between antigen and antibody is markedly increased under the condition of antigen-excess state, and therefore the MCAR may be more definitive in this condition. This explains an advantageous aspect of MCAR in comparison with the absorption-elution procedure. Lincoln and Dodd [*9*] recently reported that the elution is actually disturbed under the condition of antigen excess. Thus, nondiluted saliva and seminal fluids, which contain a great number of blood group substances to be confirmed by the classic absorption test, often give an obscure response. This difficulty can be avoided by MCAR. For secretor-type individuals, serological staining of the latent fingerprints on the paper gives, in many cases, a more distinct pattern than that of the ninhydrin test. This can be expected because the serological reaction is more sensitive than chemical methods. However, for nonsecretor-type individuals the MCAR is not as

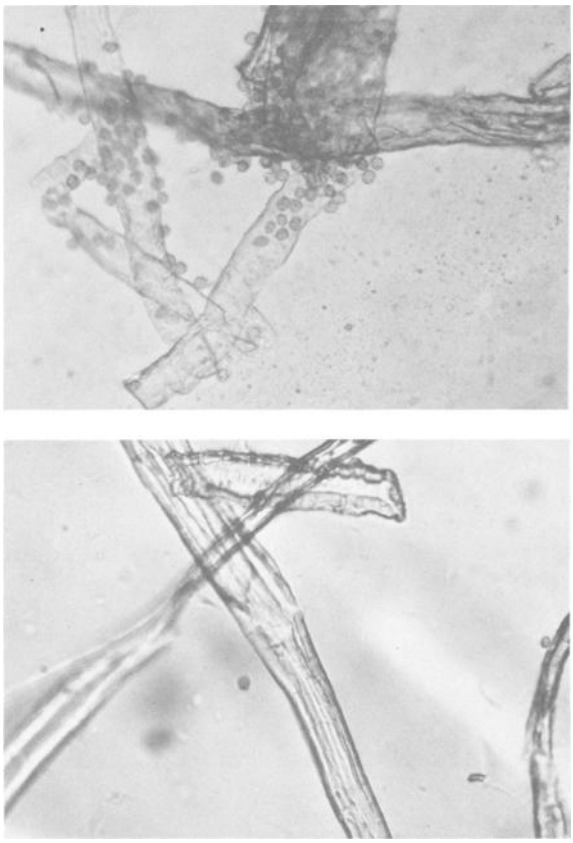

FIG. 7—*Demonstration of serological specificity of fingerprints on paper which is not impervious to water (blood type A, secretor);* (top) *sensitized with anti-A, followed by MCAR with type A cells, with positive results; and* (bottom) *sensitized with anti-B, followed by MCAR with type B cells, with negative results.*

efficient because of the insufficient amount of serologically active substances in the biological fluid of the nonsecretor. Our studies do show, however, that if one finds a trace of ninhydrin-positive response as a part of the fingerprint, the identification of blood group A-B-H is possible, irrespective of secretor type.

Blood group determination of fingerprints on solid objects such as glass have been performed by sampling them directly with an adhesive tape. These tests were occasionally unsatisfactory because of the poor sampling of the active specimens, suggesting that glycoproteins are absorbed firmly onto the glass surface [*7*]. Swabbing with absorbent cotton dipped in ethanol and water is sufficient to collect specimens from the fingerprints. More reliable results can be gained by scraping the epidermal replication with a sharp razor blade. As we have shown in Figs. 1 and 2, the patterns of MCAR findings differ considerably between both tests. The agglutination pattern obtained by swabbing is a kind of segregation, as indicated by Davidsohn [*2,3*], suggesting that blood group substances are distributed equally on the adhesive surface. This is certainly induced by the effect of the solvent. This assumption can be reasonably confirmed by the finding that the scraping method induces typical MCAR patterns, including rosette formation.

Various investigators have shown that hemagglutination is dependent not only on the number of antigenic loci but also on the distribution density of the antigens on the surface of one molecule. If the conglomerated sweat and dirt particles are examined in regard to serological specificity, they may react as a macromolecule anchored with a high density of antigenic loci. Hence, the MCAR pattern is more predominant with the scraping method than the swabbing procedure. The sensitivity of both tests is shown in Table 1.

TABLE 1—*Experimental results of blood group A-B-H determination by modified MCAR: comparison of swabbing and scraping methods.*

Blood Group	Number of Samples	MCAR from Latent Fingerprints			
		Swabbing		Scraping	
		Concordance	Discordance	Concordance	Discordance
A-Se	42	39	3[a]	40	2[a]
A-se	4	3	1[a]	3	1[a]
B-Se	28	25	3[a]	26	2[a]
B-se	7	6	1[a]	6	1[a]
AB-Se	7	7	0	6	1[a]
AB-se	2	1	1[a]	2	0
O-Se	18	17	1[b]	17	1[b]
O-se	8	7	1[c]	7	1[c]

[a] Misinterpreted as O or AB.

[b] Misinterpreted as A. The same case was misinterpreted by both methods, presumably because of contamination during sampling.

[c] Misinterpreted as weak AB, presumably because of insufficient washing after sensitization.

When both tests were performed on a complete print pattern, we found no essential differences between them. The recognition of a positive reaction with the swabbing method requires some experience with the test. Therefore, for routine examinations in which some damage to the surface of the object being examined is allowed, the scraping method is recommended as preferable to the other tests. We have shown that the initial aluminum powder procedure done by the police (a commonly practiced international rule) does not interfere with our serological examination, provided that contamination by other blood types is completely excluded. When the demonstration of fingerprints by primary criminal investigation fails, identification by blood type gives a determining clue for further investigation.

One of the useful modifications in this experiment is the use of Tween 80. This reagent prevents the nonspecific adhesion of the indicator cells so that the highly sensitive cells can be used for the test.

Finally, some cautions must be made concerning our method. Comparisons of sensitivity obtained by classic absorption, absorption-elution, and MCAR methods are shown in Table 2. The results of this comparison of techniques of morphological examination clearly indicate that the sensitivity of classic absorption is limited to macroscopic observation, that of absorption-elution is limited to light microscopy, and the MCAR responds with a sensitivity on the order of the electron microscope. The discrimination capacity of the topographical distribution of isoantigens can be utilized solely by this technique [*10*]. These advantages might be seriously negated if the modified MCAR method is performed without careful treatment of the objects to be examined. Specimens for this method must be strictly selected, and a control experiment must always be performed; otherwise, the results may lead only to confusion. Accurate observations and analyses of the results require much experience in the criminalistics and forensic fields.

TABLE 2—*Comparison of sensitivity of classic absorption, absorption-elution, and modified MCAR for the demonstration of isoantigenic activities* [4].

Technique	Maximum Detectable Dilution of Saliva	
	Secretor[a]	Nonsecretor[b]
Classic absorption (volume of antigen aliquot, 25 μl)	$1:10^2$	<1:5
Absorption-elution (volume of antigen aliquot, 1 μl)	$1:10^{4c}$	$1:10^4$
MCAR (volume of antigen aliquot, 1 μl)	$1:10^8$	$1:10^4$

[a] Saliva of blood type B was used.
[b] Saliva of blood type AB was used.
[c] Marked inhibition of elution was observed when a concentrated saliva (1:1—1:100) was examined.

Conclusion

An accurate and highly sensitive technique for the demonstration of A-B-H isoantigens from scanty trace specimens, a modification of the MCAR techniques of Kouvarik et al [*1*] and Davidsohn [*2*], was developed. With this technique, the determination of the blood group of a latent fingerprint can be performed. No highly specialized laboratory equipment is required. Fingerprints stained with ninhydrin give an excellent serological response compared to the swabbing and scraping methods also discussed. The minimal amount of latent fingerprint for the determination of serological specificity is estimated to be an 8 to 15-mm length of one epidermal ridge lining. Routine criminalistics investigations for identifying the fingerprint pattern by replication do not usually interfere with the serological investigation. The sensitivity of this test is markedly higher than that obtained by other serological examinations. The analysis of the serological findings and the conclusion with respect to the blood types should be carefully performed.

Acknowledgment

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References

[*1*] Kouvarik, S., Davidsohn, I., and Stejskal, R., "ABO Antigens in Cancer," *Archives of Pathology* (Chicago), Vol. 86, 1968, pp. 12–21.
[*2*] Davidsohn, I., "Early Immunologic Diagnosis and Prognosis of Carcinoma," *American Journal of Clinical Pathology*, Vol. 57, 1972, pp. 715–730.
[*3*] Ishiyama, I. and Okada, T., "Anwendung der modifizierten Mischzellagglutination in der forensischen Serologie; MCAR auf dem Klebebandstreifen," *Zeitschrift fuer Rechtsmedizin*, Vol. 77, 1975, pp. 25–40.
[*4*] Orui, M. and Ishiyama, I., "Evaluation of MCAR-Sensitivity in Comparison with Other Tests," *Japanese Journal of Legal Medicine*, in press.
[*5*] Okada, T. and Ishiyama, I., "Blood Group Determination Using MCAR. Blood Group Determination from Human Hairs. Summary of Reports," presented at 59th Congress of Japanese Society of Legal Medicine, Kyoto B-31, 1975.
[*6*] Okada, T. and Ishiyama, I., "Blood Group Determination Using MCAR. Blood Group Determination from Dirt and Fingerprint. Summary of Reports," presented at 59th Congress of Japanese Society of Legal Medicine, Kyoto B-32, 1975.
[*7*] Momose, T., Fukaya, T., Takagi, K., Sugimoto, T., Okada, T., and Ishiyama, I., "Blood Group Determination Using MCAR. Blood Group Determination from Latent Fingerprint.

Summary of Reports," presented at 59th Congress of Japanese Society of Legal Medicine, Kyoto B-33, 1975.

[8] Ishiyama, I., "Determination of Blood Group Specificity Using MCAR," in *Contemporary Forensic Medicine* (a textbook of forensic medicine in Japanese in cooperation with O. Prokop and W. Goehler), Igaku Shoin Co., Ltd., Tokyo, 1975, pp. 388–392.

[9] Lincoln, P. J. and Dodd, B., "The Application of Microelution Technique using Anti-Human Globulin for the Detection of the S,s,K,Fy^a,Fy^b, and JK^a Antigens in Stains," *Medicine, Science and the Law,* Vol. 15, 1975, pp. 94–101.

[10] Ishiyama, I., Nagai, T., Chang, S., and Tokoro, Y., "Topographical Determination of Transfused Cells in Incompatible Transfusion from Paraffin-Sectioned Tissue Slices," *American Journal of Clinical Pathology,* in press.

Department of Legal Medicine
Teikyo University School of Medicine
173-Tokyo, Itabashiku
Kaga 2-11-1
Japan